Plankton Biol. Ecol. 51 (2): 71–81, 2004

plankton
biology & ecology

Regional distribution of picophytoplankton in near-shore areas around Japan in early summer

AKIHIRO SHIOMOTO[1]*, HITOSHI TANAKA[2], SHINJI HASHIMOTO[3] & MITSURU YANADA[4]

[1] *National Research Institute of Fisheries Science, Fisheries Research Agency, 2–12–4, Fukuura, Kanazawa-ku, Yokohama, 236–8648 Japan*
[2] *Environmental Science Group, Environmental Health Science Laboratory, Sumitomo Chemical Co., Ltd., 2–1, Takatsukasa 4-Chome, Takarazuka, 665–8555 Japan*
[3] *Japan Science and Technology Corporation, c/o Hydrosphere Atmospheric Research Center, Nagoya University, Chikusa-ku, Furo-cho, Nagoya, 464–8601 Japan*
[4] *Hokkaido University, Minato-cho 3–1–1, Hakodate, 041–8611 Japan*

Received 22 December 2003; Accepted 20 April 2004

Abstract: We observed the regional distributions of *Synechococcus*, *Prochlorococcus* and eukaryotic picophytoplankton at the surface in near-shore areas around Japan in May and June 2000. Cell densities ranged from 10×10^2 to 10.5×10^4 cells ml^{-1} for *Synechococcus*, from 3.4 to 40.9×10^3 cells ml^{-1} for *Prochlorococcus*, except when they were absent, and from 1.4 to 95.4×10^3 cells ml^{-1} for eukaryotic picophytoplankton. The study area was roughly divided into three areas: the Kuroshio Area, the Perturbed and Oyashio Area and the Tsushima Current Area. *Synechococcus* was relatively abundant in the Kuroshio Area and the Tsushima Current Area. The cell density was positively related to phosphate concentration, suggesting that phosphate is a limiting factor for *Synechococcus*. *Prochlorochoccus* was observed in the Kuroshio Area and was relatively abundant in the East China Sea. *Prochlorochoccus* was possibly carried northward by the Kuroshio and its branches. Eukaryotic picophytoplankton were noticed to be relatively abundant in the Perturbed and Oyashio Area, especially around the fronts formed at the boundaries of subtropical water masses. It was evident that eukaryotic picophytoplankton contribute to spatial variation in the total phytoplankton biomass as well as to that with respect to the total picophytoplankton biomass.

Key words: *Synechococcus*, *Prochlorococcus*, eukaryotic picophytoplankton, around Japan, early summer

Introduction

Since the 1970s, it has become evident that picophytoplankton (<2 or 3 μm) make a significant contribution to total phytoplankton biomass in the marine ecosystem, based on data concerning size-fractionated chlorophyll *a* (Chl *a*) (Berman 1975; Bienfang 1980; Odate & Maita 1988/89; Welschmeyer et al. 1993; Odate 1996; Shiomoto & Hashimoto 2000). In near-shore areas around Japan, many studies regarding abundance, biomass and species of large-sized phytoplankton have been carried out between the 1930s and 1980s (reviewed by Terazaki 1990). Recently the significance of picophytoplankton to the total phytoplankton biomass has also been shown through observations of size-fractionated Chl *a* in near-shore areas around Japan (Maita & Odate 1988; Shimada et al. 1995; Saito et al. 1998; Hashimoto & Shiomoto 2002; Tada et al. 2003), implying the importance of picophytoplankton as primary producers in the area.

Picophytoplankton can be roughly divided into *Synechococcus*, *Prochlorococcus* and eukaryotic picophytoplankton (e.g. Jiao & Yang 1999; Partensky et al. 1999). Relationships between environmental factors and each picophytoplankton group are different: 1) *Synechococcus* are more abundant in more oligotrophic conditions than *Prochlorococcus*, 2) *Prochlorococcus* uses low irradiance more efficiently than *Synechococcus* and 3) eukaryotic picophytoplankton are more abundant in lower temperatures and more eutrophic conditions than the other picophyto-

* Corresponding author: Akihiro Shiomoto; e-mail, shiomoto@fra.affrc.go.jp

plankton (e.g. Chisholm 1992; Ishizaka et al. 1994; Jiao & Yang 1999; Partensky et al. 1999). Thus, information regarding each picophytoplankton group contributes significantly to the elucidation of the marine ecosystem in areas where picophytoplankton are significant primary producers. Studies regarding those picophytoplankton occurring in near-shore areas around Japan, however, are mostly limited to within bays (Odate 1989; Shimada et al. 1995), and studies in the East China Sea (Jiao & Yang 1999; Chang et al. 2003).

We had a chance to observe the regional distributions of the three picophytoplankton groups in near-shore areas around Japan in May and June 2000. The significant contribution of picophytoplankton to total Chl *a* has already been reported by Hashimoto & Shiomoto (2002). In this paper, we report their regional distributions in near-shore areas around Japan for the first time, and discuss the effect of some environmental factors on the picophytoplankton.

Materials and Methods

Water sampling was conducted during cruises of the R/V Daini-Kyoshin Maru in May and June 2000 (Fig. 1). Surface seawater samples were collected using an acid-cleaned plastic bucket. Flow cytometry was used for analyzing small-sized phytoplankton. For the analysis, the following fixation and preservation methods were employed. Seawater samples (10 ml) were pooled in polyethylene bottles, and fixed with 0.5% (final concentration) paraformaldehyde neutralized by potassium hydroxide. After storage for about 20 min in a refrigerator, the samples were frozen and kept at −85°C on the ship for about 40 days and at −30°C in the laboratory for about 3 days until analysis. Chemical fixation (Partensky et al. 1996), and short- and long-term storage (5–260 days) in liquid N_2 (Vaulot et al. 1989) led to a decrease in the observed picophytoplankton cell densities. The cell densities reported in this study should therefore be considered conservative estimates.

The samples were analysed with an EPICS-Elite-ESP flow cytometer (Beckman Coulter) equipped with a 15-mW argon laser exciting at 488 nm. We measured forward light scatter (FLS, an indicator of size), orange fluorescence from phycoerythrin (560–590 nm) and red fluorescence from chlorophyll (>660 nm) after excitation by 488-nm light according to Olson et al. (1993). Data were collected in listmode, and then analyzed on a personal computer using WinMDI, version 2.7, free software (Joseph Trotter). We recognized populations of various kinds of small-sized phytoplankton and estimated their equivalent spherical diameters, by comparing with 0.5, 1, 2, 3 and 6-μm sized fluorescent beads ("Fluoresbrite", Polysciences, Inc., Warrington, PA). We divided the picophytoplankton (<2-μm cell sizes) into three groups on the basis of their flow cytometric signatures: *Synechococcus*, *Prochlorococcus* and eukaryotic picophytoplankton. *Synechococcus* cells are easily recognized by the orange fluorescence of their phycoerythrin, whereas *Prochlorococcus* has smaller scatter signals than *Synechococcus* and has only red fluorescence (e.g. Olson et al. 1990). The eukaryotic picophytoplankton also have red fluorescence but have larger scatter signals than *Synechococcus*. Cell numbers were counted at a calibrated flow rate of 60 μl min^{-1} for 5 min. Five samples daily were

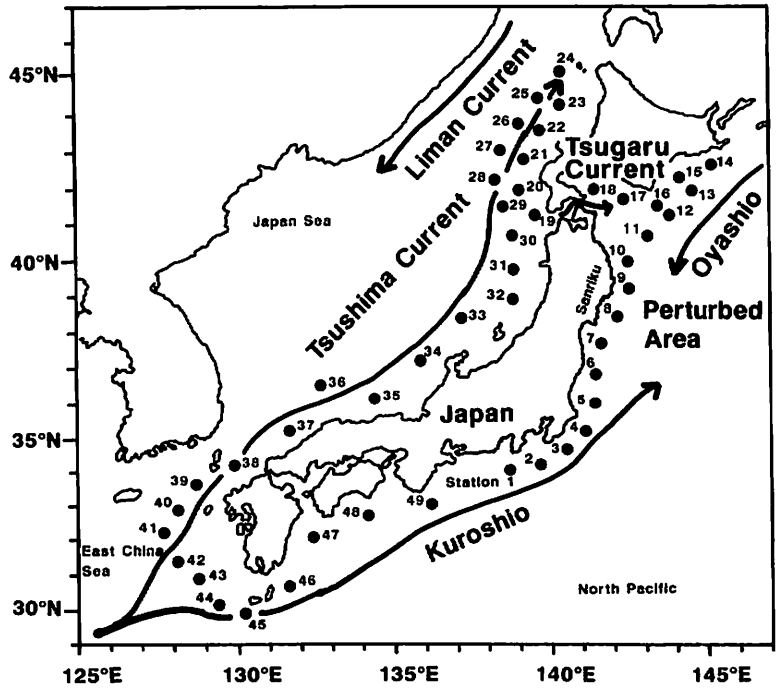

Fig. 1. Location of sampling stations (solid circles) in near-shore areas around Japan in May and June 2000, and the typical currents around Japan shown by broad arrows.

weighed before and after analysis at selected times, and the flow rate was corrected based on the difference in weight reduction between the time intervals. For *Synechococcus*, *Prochlorococcus* and eukaryotic picophytoplankton, the precision of the counting of cell density was high, with less than 4% being the coefficient of variation by three replications, using five coastal samples. Moreover, the cell number data were converted to carbon biomass using the following conversion factors (250, 53 and 2108 fgC cell^{-1} for *Synechococcus*, *Prochlorococcus* and eukaryotic picophytoplankton, respectively; Campbell et al. 1994).

In order to determine the pico-size (<2 μm size-fraction) and total Chl *a* concentration, separate seawater samples were filtered through Nuclepore filters with pore size 2 μm (>2 μm size-fraction) and Whatman GF/F filters (ca. 0.7 μm-pore size: total). Chl *a* concentration of the <2 μm size-fraction was obtained from the difference between the total and the >2 μm size-fraction. The filters were stored frozen at −20°C until analysis later on land. Chl *a* was measured with a Hitachi F-2000 fluorophotometer according to Parsons et al. (1984) for samples extracted with 90% acetone. Calibration of the fluorophotometer was performed with commercially prepared Chl *a* standard derived from Wako Pure Chemical Industries, Ltd. (Tokyo, Japan).

The surface temperature and salinity were measured with a thermometer and a Guildline salinometer (Auto Sal Model 8400A), respectively. The surface nutrient concentrations were determined by a Bran and Luebbe Auto Analyser Traacs 800 after storage at −20°C.

Results

Physical and chemical environments

Surface temperatures were mostly 10–15°C between Stns. 5 and 31 (Fig. 2A). The temperature was more than 15°C at Stns. 1–4 and Stns. 32–49. An increase in temperature from about 15 to 25°C was observed between Stns. 32 and 45.

Surface salinity was 32.4–34.2 at Stns. 5–19 and mostly less than 33.5, though the values were variable between stations (Fig. 2B). The values were almost uniform at about

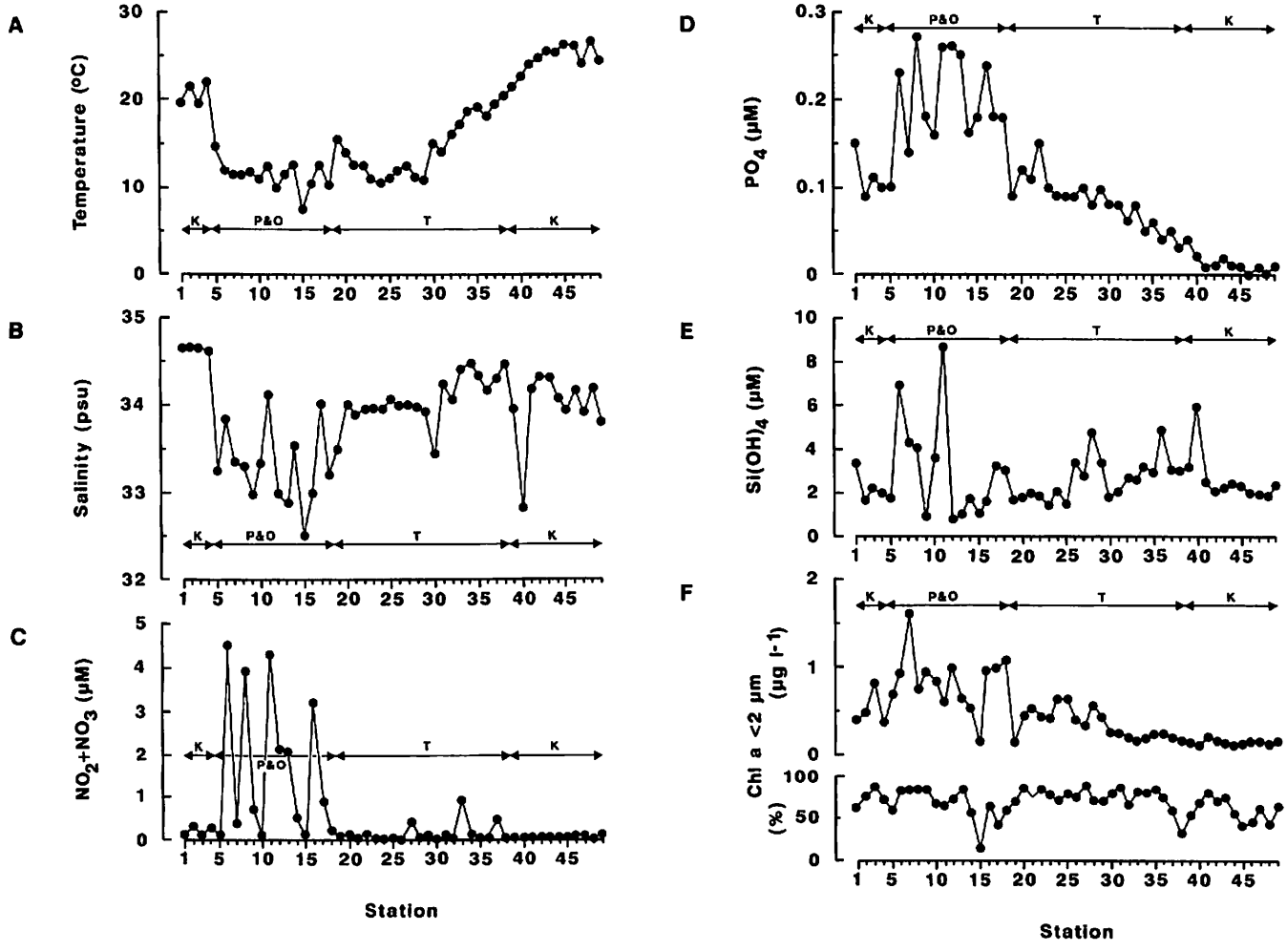

Fig. 2. Variations in temperature (A), salinity (B), concentrations of nitrite+nitrate (NO_2+NO_3) (C), silicate ($Si(OH)_4$) (D) and phosphate (PO_4) (E), and chlorophyll *a* concentration and percentage contribution of the <2 μm size-fraction (F) at the surface. K: the Kuroshio Area; P&O: the Perturbed and Oyashio Areas; T: the Tsushima Current Area.

34.7 for Stns. 1–4 and at about 34.0 for Stns. 20–29. The values varied between 33.8 and 34.5 between Stns. 30 and 49, except for the especially low values at Stns. 30 and 40.

The Kuroshio (subtropical water) and the Oyashio (subarctic water) are located on the Pacific side of the Japanese Islands (see Fig. 1). The Kuroshio and Oyashio meet off the Sanriku coast and hence this area is called the "Perturbed Area" (intermediate water between subtropical and subarctic waters) (e.g. Kawai 1972). The Kuroshio is characterized by temperatures exceeding 18°C and salinity at the surface exceeding 34.0 in May and June (Kawai 1972). Based on station location, temperature and salinity, Stns. 1–4 and 39–49 belonged to the Kuroshio Area with Stns. 39–45 being located in the East China Sea. Stns. 5–18 were classified as belonging to the Perturbed and Oyashio Areas, because it was very difficult to distinguish between stations in the Perturbed Area and the Oyashio Area based solely on temperature and salinity. On the other hand, the Tsushima Current (subtropical water) is in the Japan Sea (see Fig. 1). The Tsushima Current originates in the Kuroshio (e.g. Moriyasu 1972) and is possibly affected by the Liman Current (subarctic water) in the northern area. Stns. 19–38 thus belonged to the Tsushima Current Area and Stns. 21–29, in particular, were affected by the Liman Current, as indicated by the relatively low temperatures (Fig. 2A). In addition, salinity was remarkably low at Stns. 30 and 40 compared to those stations before and after in sequence, suggesting that the two stations were strongly affected by coastal water (Fig. 2B).

Concentrations of nitrite+nitrate (NO_2+NO_3), phosphate (PO_4) and silicate ($Si(OH)_4$) tended to be relatively high in the Perturbed and Oyashio Areas (Fig. 2C, D, E). In particular, PO_4 concentration had the characteristic of a high-north and low-south trend in the Tsushima Current Area. Relatively high NO_2+NO_3 concentrations exceeding 1 μM were observed at several stations between Stns. 6–17. The concentrations were mostly less than 0.5 μM at the remaining stations. Relatively high PO_4 concentrations, 0.14–0.27 μM, were observed at Stns. 6–17. The concentrations at other stations were less than 0.15 μM. A decreasing trend was observed for the PO_4 concentration after Stn. 18. $Si(OH)_4$ concentrations were generally between 1 and 3 μM, and relatively high values exceeding 4 μM were observed at Stns. 6–8, 11, 28, 36 and 40.

Chlorophyll *a*

Chl *a* concentrations of the <2 μm size-fraction tended to be relatively high in the Perturbed and Oyashio Areas (Fig. 2F). The Chl *a* concentrations ranged from 0.10 to 1.60 $\mu g l^{-1}$. Between Stns. 5 and 18, the concentrations exceeded 0.5 $\mu g l^{-1}$ except at Stn. 15. The concentrations were mostly less than 0.5 $\mu g l^{-1}$ at the remaining stations and, in particular, the values were less than 0.3 $\mu g l^{-1}$ after Stn. 30.

The proportion of the total Chl *a* concentration made by the <2 μm size-fraction ranged from 16 to 89% with an average share of 69% (Fig. 2F), indicating that picophytoplankton significantly contributed to the total phytoplankton biomass. Moreover, a significantly positive linear correlation was observed between the Chl *a* concentrations of the <2 μm size-fraction and the total Chl *a* concentration ($r^2=0.81$, $n=49$, $p<0.001$), indicating a significant contribution of picophytoplankton to variation in the total phytoplankton biomass.

Abundance of picophytoplankton

Synechococcus was relatively abundant in the Kuroshio Area and the Tsushima Current Area (Fig. 3A). Cell densities of *Synechococcus* ranged from 1.0×10^2 to 10.5×10^4 cells ml^{-1}. The values were markedly lower (magnitude of 10^2–10^3 cells ml^{-1}) at Stns. 5–18, especially at Stns. 8–10 and 12–16. In contrast, the cell densities were in the magnitude of 10^4 cells m^{-1} at the remaining stations except Stn.

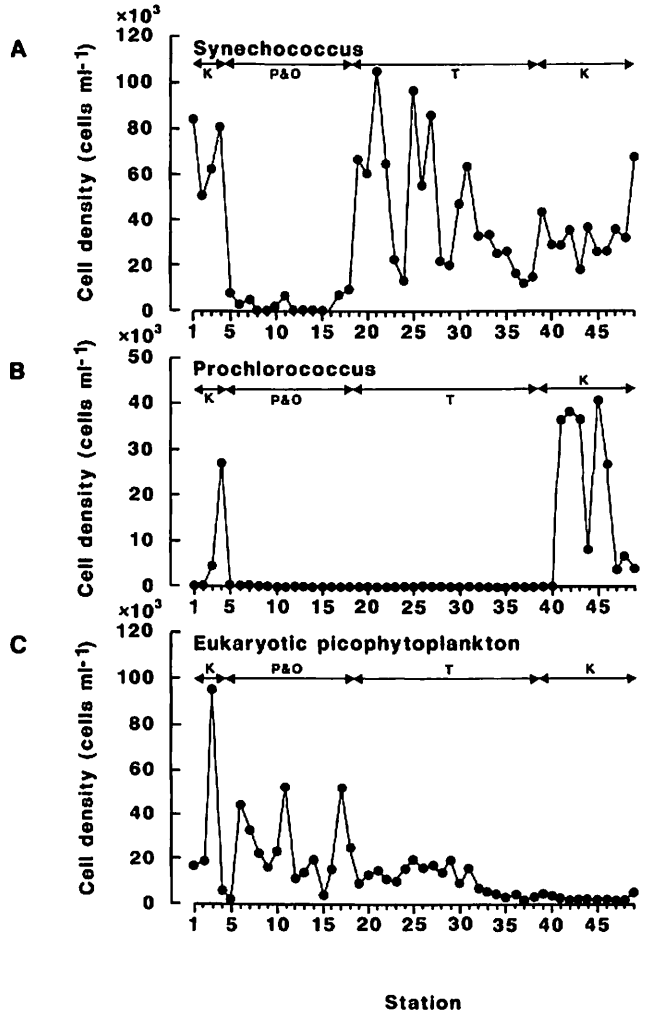

Fig. 3. Variations in cell densities of *Synechococcus* (A), *Prochlorococcus* (B) and eukaryotic picophytoplankton (C) at the surface. K: the Kuroshio Area; P&O: the Perturbed and Oyashio Areas; T: the Tsushima Current Area.

21 (magnitude of 10^5 cells ml^{-1}).

Prochlorococcus was relatively abundant in the East China Sea (Fig. 3B). *Prochrolococcus* was observed only at Stns. 3, 4 and 41–49. Cell densities of *Prochlorococcus* ranged from 3.4 to 40.9×10^3 cells ml^{-1} at these stations. Relatively high cell densities were found at Stns. 4, 41–43, 45 and 46.

Eukaryotic picophytoplankton were relatively abundant in the Perturbed and Oyashio Areas (Fig. 3C). Cell densities of eukaryotic picophytoplankton ranged from 1.4 to 95.4×10^3 cells ml^{-1}. Relatively high values exceeding 20×10^3 cells ml^{-1} were frequently observed between Stns. 6 and 18, though the maximum value was found at Stn. 3. In contrast, the cell densities were less than 20×10^3 cells ml^{-1} after Stn. 19, and the values were in the magnitude of 10^3 cells ml^{-1} after Stn. 32 in particular.

Biomass of picophytoplankton

Total picophytoplankton biomass tended to be higher in the Perturbed and Oyashio Areas (Fig. 4A). It was also found that, in the Tsushima Current Area, the values were relatively high in the northern area and low in the southern area. The biomass of *Synechococcus*, *Prochlorococcus* and eukaryotic picophytoplankton ranged from 0.1 to 26.1 $\mu gC\,l^{-1}$, from 0.2 to 2.2 $\mu gC\,l^{-1}$ except when they were absent, and from 5.3 to 201.1 $\mu gC\,l^{-1}$, respectively. Total biomass of the three plankton groups combined was between 5.3 and 217.0 $\mu gC\,l^{-1}$. The maximum value was observed at Stn. 3. Values of about 100 $\mu gC\,l^{-1}$ were observed at Stns. 6, 11 and 17, and values of 30–60 $\mu gC\,l^{-1}$ were found mostly at the remaining stations between Stns. 1–31. The values were mostly about 13 $\mu gC\,l^{-1}$ after Stn. 32.

The proportion of the total picophytoplankton biomass comprised by eukaryotic picophytoplankton was high in the Perturbed and Oyashio Areas and the Kuroshio Area, especially in the former areas (Fig. 4B). The share of *Synechococcus* was also high in the Kuroshio Area, especially in the southern area. The share of *Prochlorococchus* was rela-

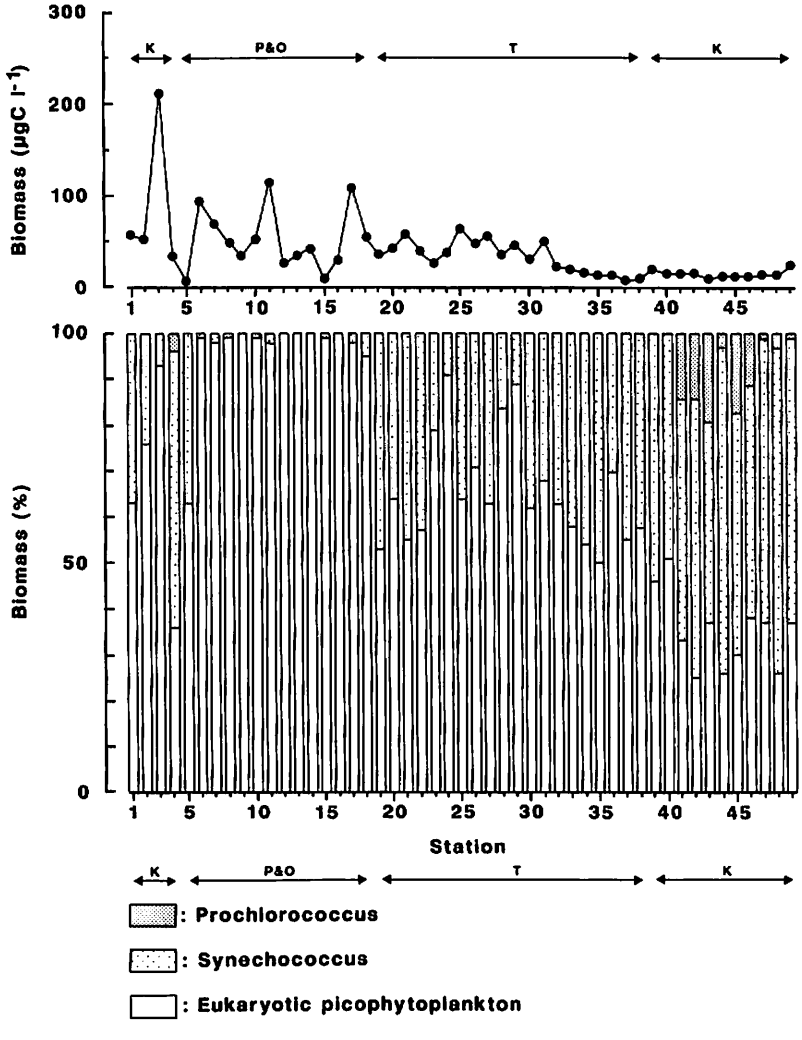

Fig. 4. Variations in total picophytoplankton biomass ($\mu gC\,l^{-1}$) (A) and the percentage composition of the total picophytoplankton biomass (B) at the surface. K: the Kuroshio Area; P&O: the Perturbed and Oyashio Areas; T: the Tsushima Current Area.

tively high in the East China Sea. Eukaryotic picophytoplankton ususlly accounted for about half or more of the total at Stns. 1–39. In particular, eukaryotic picoplankton accounted for more than 90% of the total at Stns. 6–18. *Synechococcus* was the second most abundant group after the eukaryotic picophytoplankton at most of the Stns. 1–40. At Stns. 4 and 41–49, the percentage contribution of *Synechococcus* was highest, accounting for 44–71%. The percentage contribution of *Prochlorococcus* was relatively high at Stns. 41–43, 45 and 46, accounting for 10–20%. The contribution was less than 1% at most of the remaining stations.

We analyzed the effect of *Synechococcus*, *Prochlorococcus* and eukarytotic picophytoplankton on the spatial variation in picophytoplankton biomass by performing a multiple regression analysis between the Chl *a* concentrations of the <2 μm size-fraction and the carbon biomasses of the three picophytoplankton groups. As a result, a significant relationship was observed for the regression equation ($p<0.001$), while a significant positive standard partial regression coefficient was obtained only for eukaryotic picophytoplankton ($p<0.001$). Eukaryotic picophytoplankton contributed substantially to regional variation in the Chl *a* of the <2 μm size-fraction.

Weight ratios of total picophytoplankton carbon biomass to Chl *a* concentration were calculated to be 24–276 except at Stn. 5 (6) where a low value occurred. The number of eukaryotic picophytoplankton cells at Stn. 5 was one order of magnitude lower than that at other stations with nearly equal Chl *a* concentrations in the <2 μm size-fraction as stations in the Perturbed and Oyashio Areas, implying that the low value was caused by an unknown error in analysis. Mean±standard error was calculated to be 97±7 ($n=48$) except for the above mentioned value.

Relationships between environmental factors and picophytoplankton

We investigated the relationships between physical and chemical environmental factors and the cell densities of the three picophytoplankton groups using the Spearman rank correlation test. Positive relationships were observed for the cell densities of *Synechococcus* and *Prochlorococcus* against temperature and salinity, whereas negative relationships were observed against nutrient concentrations (Table 1). Negative relationships were also observed for the cell density of eukaryotic picophytolankton against temperature and salinity, whereas positive relationships were observed against nutrient concentrations (Table 1). These results reflect the abundance of prokaryotic picophytoplankton in the subtropical waters, and the abundance of eukaryotic picophytoplankton in the subarctic waters around Japan. It was evident that picophytoplankton distribution around Japan is dependent on the water masses.

Discussion

Early summer is considered to be a transitional period from high levels to low levels, in biomass terms, of the phytoplankton community in near-shore areas around Japan. This is based on observations of seasonal variation in Chl *a* concentrations over 10 years (Imai et al. 1988). Although there is no information regarding seasonal variation in picophytoplankton abundance in near-shore areas around Japan, studies in a subarctic bay (Funka Bay; Odate 1989) and a subtropical bay (Suruga Bay; Shimada 1995) showed that picophytoplankton were abundant in spring and summer, and maximum values were found in summer. The timing of this study is thus considered to be in the period of relatively high cell abundance. On the other hand, stabilization of the water column in the upper layer progresses from spring to summer. Under stabilized conditions, picophytoplankton, especially *Prochlorococcus*, reach maximum at the subsurface (Ishizaka et al. 1994; Blanchot and Rodier 1996; Campbell et al. 1997; Maeda 2000). Hence, there is no guarantee that the cell number and species composition of picophytoplankton at the surface are representative of the entire water column. However, a indication of the areal characteristics of the picophytoplankton can be gained from observations at the surface because the distribution of picophytoplankton around Japan was characterized by the water masses.

The weight ratios of carbon biomass to Chl *a* concentration for living phytoplankton were reported to be mostly 50–200 and the mean was 128 for the surface mixed layer at station ALOHA (22°45′N, 158°W) (Campbell et al. 1994). The picophytoplankton contributed to most of the living phytoplankton carbon biomass. A similar result was found for the ratios in this study (most ratios: 30–150; mean: 97). More severe preservation conditions (quick freezing in liquid N_2 and storage at −70°C until analysis) compared to those in this study were adopted in the former study. This thus implies that the cell density values of the three picophytoplankton groups measured after freezing in

Table 1. Spearman rank correlation coefficients between cell densities of the three picophytoplankton groups and environmental factors.

	Temperature	Salinity	NO_2+NO_3	PO_4	$Si(OH)_4$
Synechococcus	0.44**	0.47***	−0.43**	−0.44**	−0.07
Prochlorochoccus	0.70***	0.32*	−0.29*	−0.58***	−0.12
Eukaryotic picophytoplankton	−0.65***	−0.20	0.51***	0.76***	0.18

*$p<0.05$; **$p<0.01$; ***$p<0.001$.

this study are reliable on the whole.

The magnitudes of the cell densities of the three picophytoplankton groups obtained in this study are nearly equal to those reported from the offshore North Pacific and the East China Sea. Picophytoplankton abundance in near-shore areas around Japan in early summer is neither considerably higher nor lower than that in the offshore area or in a marginal sea. The cell densities of *Synechococcus* and eukaryotic picophytoplankton were in the magnitude of 10^2–10^5 cells ml^{-1} (mostly in the magnitude of 10^3–10^4 cells ml^{-1}) (Fig. 3A, C). The maximum cell density of *Prochlorococcus* was 4.1×10^4 cells ml^{-1} (Fig. 3B). Cell densities reported previously in the surface waters of the offshore subtropical and subarctic North Pacific in summer were in the magnitude of 10^2–10^5 cells ml^{-1} for *Synechococcus*, and in the magnitude of 10^2–10^4 cells ml^{-1} for eukaryotic picophytoplankton (Odate et al. 1990; Blanchot et al. 1992; Ishizaka et al. 1994; Shimada et al. 1996; Campbell et al. 1997; Maeda 2000). The maximum cell density of *Prochlorococcus* was in the magnitude of 10^4–10^5 cells ml^{-1} (Ishizaka et al. 1994; Shimada et al. 1996; Campbell et al. 1997). The cell densities of the three picophytoplankton groups in the winter (Jiao & Yang 1999) and those of *Synechococcus* in each season (Chang et al. 2003) in the East China Sea were within the ranges of those in the summertime offshore in the North Pacific.

We converted cell densities to carbon biomass using the factors applied previously by Campbell et al. (1994) in the North Pacific, because the present study was also carried out in this region. However, the same conversion factors have not always been used for estimating the picophytoplankton carbon biomass. Nevertheless, the factors for *Prochlorococcus* and eukaryotic piophytoplankton previously in use are similar: the values are about 50 fgC $cell^{-1}$ for the former and about 2000 fgC $cell^{-1}$ for the latter (Li et al. 1992; Campbell et al. 1994; Zubkov et al. 1998; DuRand et al. 2001; Bertilsson et al. 2003). In contrast, the factors for *Synechococcus* have mostly fluctuated between 92 and 250 fgC $cell^{-1}$ depending on the researchers (Li et al. 1992; Campbell et al. 1994; Zubkov et al. 1998; DuRand et al. 2001; Bertilsson et al. 2003). The factor used in the present study (Campbell et al.'s factor) is the maximum value previously reported. Hence, we estimated the total carbon biomass of the three picophytoplankton groups and the percentage contribution of *Synechococcus* using the minimum conversion factor for *Synechococcus* (92), and then compared the total biomass and the contribution estimated by the maximum factor. As a result, the total carbon biomasses of the three groups combined estimated by the minimum factor are an average of 81% (range of 55–100%) of those estimated by the maximum factor, showing no large difference between the biomasses estimated by the maximum and minimum factors. On the other hand, the percentage contributions of *Synechococcus* estimated by the minimum factor are no more than 50% of those estimated by the maximum factor (range of 0–48%; mean of 16%). Stations where *Synechococcus* comprised the majority of the total carbon biomass were not found, which is a very different result to that obtained by using the maximum factor (percentage contributions exceeded 50% at most stations in the Kuroshio Area; see Fig. 4). It is imperative that the most suitable factor for use around Japan be determined.

Synechococcus was observed at every station and was relatively abundant in the Kuroshio Area and the Tsushima Current Area (Fig. 3A). The Tsushima Current originates in the Kuroshio (e.g. Moriyasu et al. 1972). Accordingly, *Synechococcus* was characterized as a group exhibiting relative abundance in areas originating in the Kuroshio around Japan.

Cell densities of *Synechococcus* were high in the northern area of the Tushima Current Area (Fig. 3A). *Synechococcus* tends to be more abundant in water of higher temperature (e.g. Murphy & Haugen 1985; Waterbury et al. 1986). However, surface temperatures were lower in the northern area of the Tsushima Current Area than in the southern area and in the Kuroshio Area (Fig. 2A), implying that temperature was not the main factor causing the relatively high cell densities in the northern area of the Tsushima Current Area. On the other hand, nitrogenous nutrient (NO_3) concentrations have been shown to be an important factor in determining the biomass and growth rate of *Synechococcus* (Blanchot et al. 1992; Chang et al. 2003). Hence, we analyzed the relationships between nutrient concentrations and the cell density of *Synechococcus* in the Kuroshio Area and the Tsushima Current Area, using the Spearman rank correlation test. As a result, a significant relationship was not found between NO_2+NO_3 concentration and the cell density of *Synechococcus*. On the other hand, a significantly positive relationship was observed between PO_4 concentration and cell density (Fig. 5). It is thus possible that PO_4 is a limiting factor for *Synechococcus* growth in waters originating in the Kuroshio around Japan. NO_2+NO_3 was nearly exhausted at most stations in the two areas (Fig. 2C). However, picophytoplankton have been shown to preferentially use ammonia (e.g. Probyn 1985; Probyn & Painting 1985) and some *Synechococcus* seem to be able to fix atmospheric nitrogen (Mitsui et al. 1986; Sachs & Repeta 1999). The nitrogen requirements of picophytopankton may be fulfilled by ammonia or N_2.

Moutin et al. (2002) suggested that *Synechococcus* has a high affinity for phosphate and a high maximum uptake rate of phosphate, and thus *Synechococcus* is usually the dominant picophytoplankton group in the Mediterranean Sea with its phosphorous-depleted environments. This characteristic of *Synechococcus* seems contrary to that obtained in this study. Several populations are observed for *Synechococcus* with regard to nutrient conditions (see Partensky et al. 1999). Examining Fig. 5 carefully, the cell densities of *Synechococcus* were nearly uniform at less than 0.07 μM of PO_4, and the increasing trend of cell densities was found to begin at concentrations higher than 0.07 μM of PO_4. This suggests that there are two populations of *Synechococcus* in

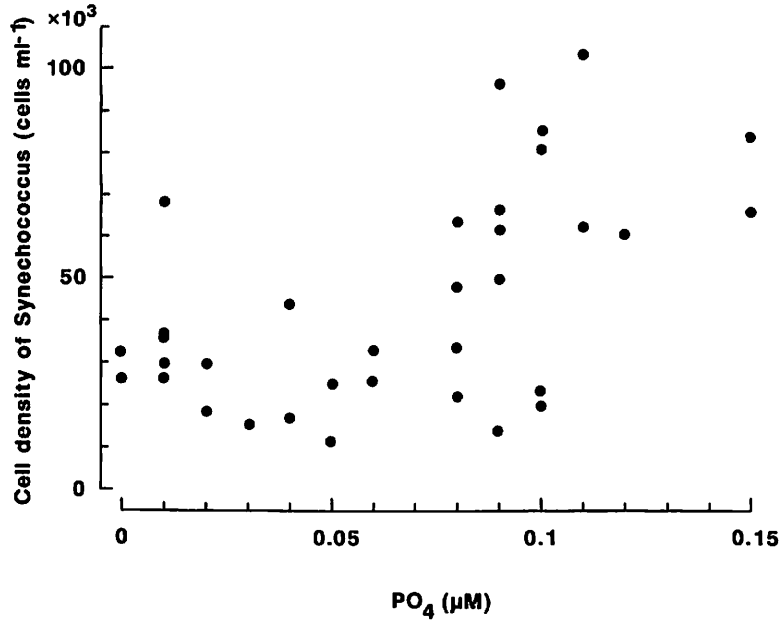

Fig. 5. Relationship between phosphate (PO_4) concentration and cell density of *Synechococcus* at the surface in the Kuroshio Area and the Tsushima Current Area. Spearman rank correlation coefficient (r_s)=0.44, n=35, p<0.01.

the Kuroshio Area and the Tsushima Current Area: one is adapted to oligotrophic conditions and the other to mesotrophic conditions. Phosphate limitation is considered to be one of the characteristics of the latter population.

Prochlorococcus was observed only at Stns. 3, 4 and 41–49, especially at Stns. 4, 41–43, 45 and 46 (Fig. 3B). These stations were located in the Kuroshio Area and, in particular, the stations with relatively high cell densities (Stns. 41–43 and 45) were located in the East China Sea. The Kuroshio water passes through the East China Sea and then goes north along the coast of Japan in the Pacific Ocean (see Fig. 1). *Prochlorococcus* in near-shore areas around Japan is thus probably carried northward by the Kuroshio and its branches.

On the other hand, on the Japan Sea side the Tsushima Current water, originating in the Kuroshio water, goes north along the coast of Japan (see Fig. 1). Accordingly, it was natural to expect that *Prochlorococcus* would be observed even in the Tsushima Current Area. However, *Prochlorococcus* was not observed in this area (Fig. 3B). Temperature plays a limiting role in the growth of *Prochlorococcus* when it is low at <15–18°C, and has an inhibiting role below 10°C (Olson et al. 1990; Buck et al. 1996), illustrating the important role of temperature in the distribution of *Prochlorococcus*. Temperatures mostly exceeded 20°C in the Kuroshio Area, whereas temperatures were less than 18°C in the Tsushima Current Area outside of the southern stations (Stns. 34–38) (Fig. 2C). The non-occurrence of *Prochlorococcus* in the Tsushima Current Area can thus be attributed principally to growth limitation due to the relatively low temperatures. Moreover, *Prochlorococcus* were not observed at Stns. 34–38 located in the Tsushima Current Area and Stns. 39 and 40 located in the Kuroshio Area (Fig. 3B), though temperatures were more than 18°C at these stations (Fig. 2C). Temperature may thus be a necessary factor but not necessarily the controlling factor affecting *Prochlorococcus* distribution.

This study shows that eukaryotic picophytoplankton contribute to the spatial variation in the total picophytoplankton biomass. Based on the results of the same shipboard observations, Hashimoto & Shiomoto (2002) stated that picophytoplankton significantly contribute to regional variation in the total phytoplankton biomass in near-shore areas around Japan. Consequently, it is evident that eukarytotic picophytoplankton contribute to spatial variation in the total phytoplankton biomass in near-shore areas around Japan in early summer.

Relatively high cell densities of eukaryotic picophytoplankton were observed in the Perturbed and Oyashio Areas (Stns. 5–18), especially at Stns. 6, 11 and 17 (Fig. 3C). Relatively high salinities were also noticed at these three stations (Fig. 2B). Moreover, a significantly positive linear relationship was observed between salinity and cell density in the Perturbed and Oyashio Areas (Fig. 6A). Two subtropical water masses (the Kuroshio water and the Tsugaru Current water) with relatively high salinity abut the Perturbed and Oyashio Areas (e.g. Kawai 1972; see Fig. 1). Thus, in the Perturbed and Oyashio Areas (Stns. 5–18), the higher the salinity at the station, the nearer the station was located to the front between those subtropical waters and the Perturbed and Oyashio Areas. Accordingly, eukaryotic picophytoplankton tend to be relatively abundant in the Perturbed and Oyashio Areas around Japan, especially around the fronts.

The fronts in the Perturbed and Oyashio Areas are considered to be affected by warm water masses. Indeed, the

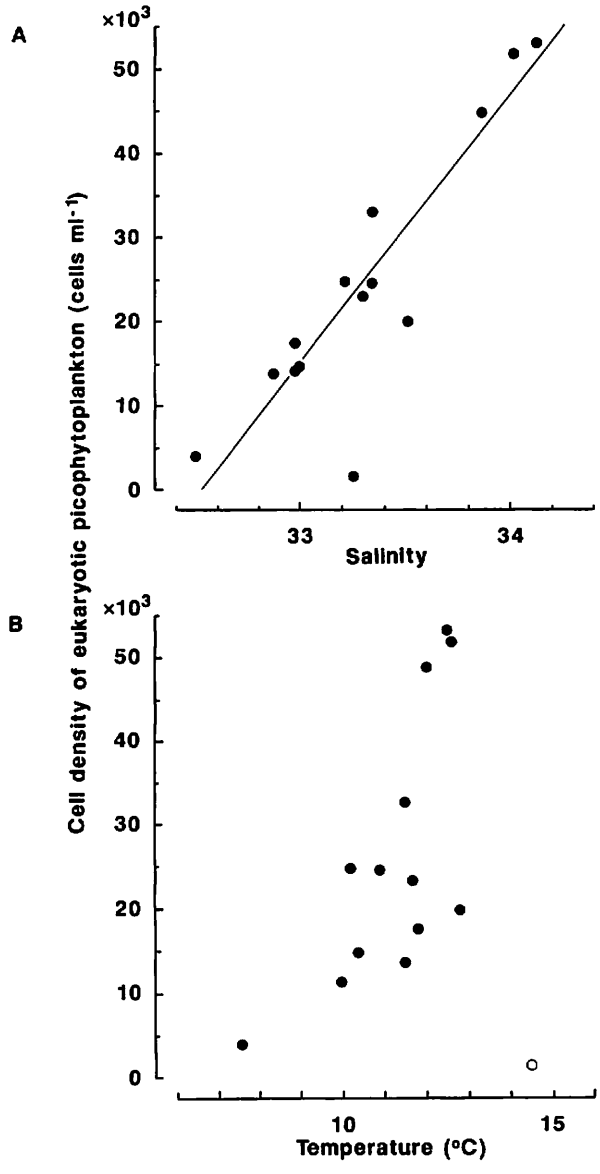

Fig. 6. Relationship between salinity (A) and temperature (B), and cell density of eukaryotic picophytoplankton at the surface in the Perturbed and Oyashio Areas. In Fig. 6A, the solid line indicates a regression line: $Y=(31.7X-1030.9)\times10^3$ ($r^2=0.77$, $n=14$, $p<0.0001$): X: salinity; Y: cell density. In Fig. 6B, Spearman rank correlation coefficient (r_s) except the value at Stn. 5 (open circle)=0.61, $n=13$, $p<0.05$.

temperatures at Stns. 6, 11 and 17, which were located around the front, are somewhat higher than those at most other stations in the area (Fig. 2a). Field studies have shown that picophytoplankton productivity and growth rates increase with a rise in temperature (Shiomoto et al. 1997; Furnas & Crosbie 1999). A significantly positive relationship was actually observed between temperature and the cell densities of eukaryotic picophytoplankton in the Perturbed and Oyashio Areas, except at Stn. 5 where a serious error in analysis may have occurred (Fig. 6B). Nutrients were not depleted in the area except for NO_2+NO_3 at some stations (Fig. 2C, D, E). Accordingly, the somewhat higher temperatures are possibly responsible for a part of the relatively high productivity of eukaryotic picophytoplankton around the fronts and hence the relatively high cell densities.

The maximum cell density of eukaryotic picophytoplankton was found at Stn. 3 (Fig. 3C). Stn. 3 was located around the front between the coastal and the Kuroshio waters off the Boso Peninsula (Fig. 1; Anonymous 2000). The temperature as well as the nutrient concentrations at Stn. 3 were not substantially different from those at the other stations (Stns. 1, 2 and 4) close to Stn. 3 (Fig. 2A, C, D, E). This indicates that temperature is not responsible for the relatively high cell densities. From the above observations, eukaryotic picophytoplankton seem to be present at relatively high densities around fronts in near-shore areas around Japan, though the factors leading to those relatively high densities may differ between areas.

Acknowledgement

We are extremely grateful to the captain and crew of the R/V Daini-Kyoshin Maru for their assistance with sample collection during the cruise.

Literature Cited

Anonymous 2000. Information on fisheries and oceanographic condition (Taiheiyo gyokaikyo sokuho). No. 1686. JAFIC. (in Japanese)

Berman, T. 1975. Size fractionation of natural aquatic populations associated with autotrophic and heterotrophic carbon uptake. *Mar. Biol.* **33**: 215–220.

Bertilsson, S., O. Berglund, D. M. Karl & S. W. Chisholm 2003. Elemental composition of marine *Prochlorococcus* and *Synechococcus*: Implications for the ecological stoichiometry. *Limnol. Oceanogr.* **48**: 1721–1731.

Bienfang, P. K. 1980. Phytoplankton sinking rates in oligotrophic waters off Hawaii, USA. *Mar. Biol.* **61**: 69–77.

Blanchot, J. & M. Rodier 1996. Picophytoplankton abundance and biomass in the western tropical Pacific Ocean during the 1992 El Niño year: results from flow cytometry. *Deep-Sea Res. I* **43**: 877–895.

Blanchot, J., M. Rodier & A. Le Bouteiller 1992. Effect of El Niño Southern Oscillation events on the distribution and abundance of phytoplankton in the Western Pacific Tropical Ocean along 165°E. *J. Plankton Res.* **14**: 137–156.

Buck, K. R., F. P. Chavez & L. Campbell 1996. Basin-wide distributions of living carbon components and the inverted trophic pyramid of the central gyre of the North Atlantic Ocean, summer 1993. *Aquat. Microb. Ecol.* **10**: 283–298.

Campbell, L., H. Liu, H. A. Nolla & D. Vaulot 1997. Annual variability of phytoplankton and bacteria in the subtropical North Pacific Ocean at Station ALOHA during the 1991–1994 ENSO event. *Deep-Sea Res. I* **44**: 167–192.

Campbell, L., H. A. Nolla & D. Vaulot 1994. The importance of *Prochlorococcus* to community structure in the central North

Pacific. *Limnol. Oceangr.* **39**: 954–961.

Chang, J., K.-H. Lin, K.-M. Chen, G.-C. Gong & K.-P. Chiang 2003. *Synechococcus* growth and mortality rates in the East China Sea: range of variations and correlation with environmental factors. *Deep-Sea Res. II* **50**: 1265–1278.

Chisholm, S. W. 1992. Phytoplankton size, p. 213–237. In *Primary Productivity and Biogeochemical Cycles in the Sea* (eds. Falkowski, P. G. & A. D. Woodhead). Plenum, New York.

DuRand, M. D., R. J. Olson & S. W. Chisholm 2001. Phytoplankton population dynamics at the Bermuda Atlantic Time-series station in the Sargasso Sea. *Deep-Sea Res. II* **48**: 1983–2003.

Furnas, M. & N. D. Crosbie 1999. In situ growth dynamics of the photosynthetic prokaryotic picoplankters *Synechococcus* and *Prochlorococcus*, p. 387–417. In *Marine Cyanobacteria* (eds. Charpy, L. & A. W. D. Larkum). Bulletin de l'Institut oceanographique, Monaco, numero special 19.

Hashimoto, S. & A. Shiomoto 2002. Regional distribution of size-fractionated chlorophyll *a* concentration at the sea surface water adjacent to Japan in May–June 2000. *Bull. Jpn. Soc. Fish. Oceanogr.* **66**: 148–154. (in Japanese with English abstract)

Imai, M., S. Ebara, K. Kawashima, N. Kubo, N. Sato & E. Moriyama 1988. Seasonal variation of chlorophyll-a in the seas around Japan. *Oceanogr. Mag.* **38**: 23–32.

Ishizaka, J., H. Kiyosawa, K. Ishida, K. Ishikawa & M. Takahashi 1994. Meridional distribution and carbon biomass of autotrophic picoplankton in the Central North Pacific Ocean during late northern summer 1990. *Deep-Sea Res.* **41**: 1745–1766.

Jiao, N. & Y. Yang 1999. Distribution of *Synechococcus*, *Prochlorococcus*, and picoeukaryotes in the East China Sea, p. 435–441. In *Marine Cyanobacteria* (eds. Charpy, L. & A. W. D. Larkum). Bulletin de l'Institut oceanographique, Monaco, numero special 19.

Kawai, H. 1972. Hydrography of the Kuroshio Extension, p. 235–352. In *Kuroshio, its Physical Aspects* (eds. Stommel, H., & K. Yoshida). Univ. Tokyo Press, Tokyo.

Li, W. K. W., P. M. Dickie, B. D. Irwin & A. M. Wood 1992. Biomass of bacteria, cyanobacteria, prochlorophytes and photosynthetic eukaryotes in the Sargasso Sea. *Deep-Sea Res.* **39**: 501–519.

Maeda, H. 2000. Ecology of marine picophytoplankton, p. 141–147. In *Marine Microorganisms* (ed. Ohwada, K.). Kaiyo Monthly, special edition, 23, Kaiyo Shuppan, Tokyo.

Maita, Y. & T. Odate. 1988. Seasonal changes in size-fractionated primary production and nutrient concentrations in the temperate neritic water of Funka Bay, Japan. *J. Oceanogr. Soc. Japan* **44**: 268–279.

Mitsui, A., S. Kumazawa, A. Takahashi, H. Ikemoto, S. Cao & T. Arai 1986. Strategy by which nitrogen-fixing unicellular cyanobacteria grow photoautotrophically. *Nature* **323**: 720–722.

Moriyasu, S. 1972. The Tsushima Current, p. 353–369. In *Kuroshio, its Physical Aspects* (eds. Stommel, H. & K. Yoshida). Univ. Tokyo Press, Tokyo.

Moutin, T., T. F. Thingstad, F. V. Wambeke, D. Marie, G. Slawyk, P. R. Raimbault & H. Claustre 2002. Does competition for nanomolar phosphate supply explain the predominance of the cyanobacterium *Synechococcus*? *Limnol. Oceanogr.* **47**: 1562–1567.

Murphy L. S. & E. M. Haugen 1985. The distribution and abundance of phototrophioc ultraplankton in the North Atlantic. *Limnol. Oceanogr.* **30**: 47–58.

Odate, T. 1989. Seasonal changes in cell density of cyanobacteria and other picophytoplankton populations in Funka Bay, Japan. *Bull. Plankton Soc. Japan* **36**: 53–61.

Odate, T. 1996. Abundance and size composition of the summer phytoplankton communities in the western North Pacific Ocean, the Bering Sea, and the Gulf of Alaska. *J. Oceanogr.* **52**: 335–351.

Odate, T. & Y. Maita 1988/89. Regional variation in the size composition of phytoplankton communities in the western North Pacific Ocean. *Biol. Oceanogr.* **6**: 65–77.

Odate, T., M. Yanada, L. V. Castillo & Y. Maita 1990. Distribution of cyanobacteria and other picophytoplankton in the western North Pacific Ocean, summer 1989. *J. Oceanogr. Soc. Japan* **46**: 184–189.

Olson, R. J., S. W. Chisholm, E. R. Zettler, M. A. Altabet & J. A. Dusenberry 1990. Spatial and temporal distributions of prochlorophyte picoplankton in the North Atlantic Ocean. *Deep-Sea Res.* **37**: 1033–1051.

Olson, R. J., E. R. Zettler & M. D. DuRand 1993. Phytoplankton analysis using flow cytometry, p. 175–186. In *Handbook of Methods in Aquatic Microbial Ecology* (eds. Kemp, P. F., B. F. Sherr & J. J. Cole). Lewis Publishers, Florida.

Parsons, T. R., Y. Maita & C. M. Lalli 1984. *A Manual of Chemical and Biological Methods for Seawater Analysis*. Pergamon Press, Oxford, 173 pp.

Partensky, F., J. Blanchot, F. Lantoine, J. Neveuz & D. Marie 1996. Vertical structure of picophytoplankton at different trophic sites of the tropical northeastern Atlantic Ocean. *Deep-Sea Res. I* **43**: 1191–1213.

Partensky, F., J. Blanchot & D. Vaulot 1999. Differential distribution and ecology of *Prochlorococcus* and *Synechococcus* in oceanic waters: a review, p. 457-475. In *Marine Cyanobacteria* (eds. Charpy, L. & A. W. D. Larkum). Bulletin de l'Institut oceanographique, Monaco, numero special 19.

Probyn, T. A. 1985. Nitrogen uptake by size-fractionated phytoplankton populations in the southern Benguela upwelling system. *Mar. Ecol. Prog. Ser.* **22**: 249–258.

Probyn, T. A. & S. J. Painting 1985. Nitrogen uptake by size-fractionated phytoplankton populations in Antarctic surface waters. *Limnol. Oceanogr.* **30**: 1327–1332.

Sachs, J. P. & D. J. Repeta 1999. Oligotrophy and nitrogen fixation during eastern Mediterranean sapropel events. *Science* **286**: 2485–2488.

Saito, H., H. Kasai, M. Kashiwai, Y. Kawasaki, T Kono, S. Taguchi & A. Tsuda 1998. General description of seasonal variations in nutrients, chlorophyll *a*, and netplankton biomass along the A-line transect, western subarctic Pacific, from 1990 to 1994. *Bull. Hokkaido Nat. Fish. Res. Inst.* **62**: 1–62.

Shimada, A., T. Maruyama & S. Miyachi 1996. Vertical distributions and photosynthetic action spectra of two oceanic picophytoplankters, *Prochlorococcus marinus* and *Synechococcus* sp. *Mar. Biol.* **127**: 15–23.

Shimada, A., M. Nishijima & T. Maruyama 1995. Seasonal appearance of *Prochlorococcus* in Suruga Bay, Japan in 1992–1993. *J. Oceanogr.* **51**: 289–300.

Shiomoto, A. & S. Hashimoto 2000. Comparison of east and west

chlorophyll *a* standing stock and oceanic habitat along the Transition Domain of the North Pacific. *J. Plankton Res.* **22**: 1–14.

Shiomoto, A., K. Tadokoro, K. Monaka & M. Nanba 1997. Productivity of picoplankton compared with that of larger phytoplankton in the subarctic region. *J. Plankton Res.* **19**: 907–916.

Tada, K., K. Ichimi & T. Hashimoto 2003. Size fractionations of phytoplankton community in Osaka Bay and Kii Channel. *Bull. Coast. Oceanogr.* **40**: 197–204. (in Japanese with English abstract)

Terazaki, M. 1990. Plankton in the water adjacent to Japan, p. 265–281. In *Coastal Oceanography of Japanese Islands, supplementary volume* (ed. Coastal Oceanography Research Committee, the Oceanographical Society of Japan). Tokai Univ. Press, Tokyo. (in Japanese)

Vaulot, D., C. Courties & F. Partensky 1989. A simple method to preserve oceanic phytoplankton for flow cytometry analyses. *Cytometry* **10**: 629–635.

Waterbury, J. B., S. W. Watson, F. W. Valois & D. G. Franks 1986. Biological and ecological characterization of the marine unicellular cyanobacterium *Synechococcus*, p. 71–120. In *Photosynthetic Picoplankton* (eds. Platt, T. & W. K. W. Li). Can. Bull. Fish. Aquat. Sci. 214.

Welschmeyer, N. A., S. Strom, R. Goericke, G. DiTullio, M. Belvin & W. Peterson 1993. Primary production in the subarctic Pacific Ocean: Project SUPER. *Prog. Oceanogr.* **32**: 101–135.

Zubkov, M. V., M. A. Sleigh, G. A. Tarran, P. H. Burkill & R. J. G. Leakey 1998. Picoplankton community structure on an Atlantic transect from 50°N to 50°S. *Deep-Sea Res. I* **45**: 1339–1355.